Int J Clin Exp Pathol 2015;8(4):4044-4053
www.ijcep.com /ISSN:1936-2625/IJCEP0002061

Original Article

Relationship between MLH-1, MSH-2, PMS-2, MSH-6 expression and clinicopathological features in colorectal cancer

Birgül Karahan[1], Asuman Argon[1], Mehmet Yıldırım[2], Enver Vardar[1]

[1]Department of Pathology, Izmir Bozyaka Training and Research Hospital, Izmir, Turkey; [2]Department of General Surgery, Izmir Bozyaka Training and Research Hospital, Izmir, Turkey

Received August 23, 2014; Accepted September 25, 2014; Epub April 1, 2015; Published April 15, 2015

Abstract: Colorectal cancers are the third most common in both sexes and they are the second most common cause of cancer-related death. 12-15% of colorectal cancers develop through microsatellite instability (the hereditary mutation in at least one of DNA mismatch repair genes) pathway and they are 2-5% hereditary. In this study, we investigated the correlation between the clinicopathological features themselves and also the correlation between them and the immunohistochemical MLH-1, MSH-2, PMS-2, MSH-6 expressions in a total of 186 resection materials with colorectal adenocarcinoma between 2008 and 2012. All the cases were retrospectively evaluated in terms of age, sex, localization, size, accompanying polyp, multiple tumor, arising from polyp, differentiation, mucinous differentiation, pathological tumor stage, lymphovascular and perineural invasion, lymphocyte amount in the tumor microenvironment, surgical border and lymph node metastasis. We prepared multiple tissue blocks which had 4-millimeter tumor. Immunohistochemically, MLH-1, MSH-2, PMS-2, MSH-6 primary antibodies were studied. Statistically, "Kruskal-Wallis" ve "Pearson's chi-squared" tests were used. We found a positive correlation between loss of MLH-1 and PMS-2 expressions and the right-colon location, poor and mucinous differentiation and dense lymphocytic infiltration. In addition, loss of MSH-2 and MSH-6 expressions was correlated with the right-colon location, poor and mucinous differentiation. We found a meaningful relationship between immunohistochemical markers and clinicopathological features usually observed in tumors with microsatellite instability. This finding may arouse suspicion for MSI. However, the findings in our study must be supported with studies conducted in large series including molecular methods.

Keywords: Microsatellite instability, colorectal cancer, MLH-1, MSH-2, PMS-2, MSH-6

Introduction

Of all the cancers in the world, colorectal cancer (CRC) is the third most common cancer [1]. While the mean age is 62, the risk group range is 60-79 [2]. The incidence under 50 is below 20% unless there are predisposing factors [3]. About 75% of the CRCs occur sporadically and they are inherited in 5% [4]. As known, malignancies occur due to the accumulation of mutations in genes that directly control cell growth and death. CRCs are thought to develop from two pathways which are defined as chromosomal instability (CIN) and microsatellite instability (MSI) [5]. Although most of the CRCs which develop from CIN are sporadic, Familial Adenomatous Polyposis (FAP) and MUTYH-associated polyposis (MAP) developing from this pathway are familial and each of them constitutes 1% of CRCs [6]. The existence of noteworthy features in clinical presentation makes it easy to scan and detect these syndroms.

MSI is characterized by small deletions or insertions within a number of repeated nucleotide units in DNA due to defects in DNA mismatch repair process [7, 8]. Although MSI pathway is detected in 12-15% of CRCs, 2-5% of them is hereditary [3]. The hereditary mutation of one of the five DNA mismatch repair genes (MSH-2, MSH-6, MLH-1, PMS-1 ve PMS-2) results in Lynch syndrome/Hereditary Nonpolyposis Colon Cancer (HNPCC). Tumors that arise via the MSI show certain clinicopathological fea-

tures including proximal colon location, mucinous histology, and infiltration by lymphocytes [5].

Detecting tumors of hereditary and/or tumors with MSI is important because of differences in therapy and in order to follow the other family members before they contract cancer. The genetic analysis is the gold standard for the diagnosis of hereditary tumors. However, the genetic analysis is difficult and expensive so it cannot be used for screening of all colorectal tumors [9-11]. Thus, the clinicopathological and immunohistochemical features that will be used in the selection of the patients to be referred to genetic analysis must be clarified.

In this study, we investigated the correlation between the clinicopathological features themselves (age, sex, localization, size of tumor, accompanying polyp, multiple tumor, arising in polyp, differentiation, mucinous component, pathologic stage of tumor, lymphovascular and perineural invasion, lymphocyte density in tumor microenvironment, surgical margin and lymph node metastasis) and also the correlation between them and the immunohistochemical MLH-1, MSH-2, PMS-2, MSH-6 expressions in a total of 186 resection materials with colorectal adenocarcinoma between 2008 and 2012.

Materials and methods

A total of 186 patients who underwent resection for colorectal adenocarcinoma in our institution from 2008 to 2012 were included in this study. The clinical features of the cases and the macroscopic features of the tumors were obtained from the hospital archive system.

Hematoxylin-Eosin-stained sections of the 186 patients were reevaluated by at least two pathologists in terms of differentiation, mucinous component, arising in polyp, pathologic stage of tumor, lymphovascular and perineural invasion, lymphocyte density in tumor microenvironment, surgical margin and lymph node metastasis.

The localization was classified as cecum, ascending colon, transverse colon, descending colon, sigmoid colon, rectosigmoid junction and rectum; the growth pattern as ulcerovegetative, polypoid and ulcerous. For the histological differentiation degree and pathological tumor stage, 2010 World Health Organization Classification of Tumours of the Digestive System was used [12]. The lymphocyte density in tumor microenvironment was divided into three categories as: 1; stromal lymphocytic infiltration, 2; stromal lymphocyte infiltration and glandular infiltration, 3; Crohn-like lymphocytic infiltration regardless of the lymphocytic infiltration in the tumor. The presence of macroscopic and/or microscopic tumor at the surgical margin was considered as surgical margin positivity. The other parameters were classified as present or absent.

Immunohistochemistry

One of the blocks that had gone through the routine process and reflected the tumor features best was selected. After the tumor area adjacent to normal mucosa and/or lymphocytic infiltration was marked, 4 mm paraffinized tissue was removed and the multiple tissue blocks were prepared (**Figure 1**). 4-micron-thick sections were obtained for IHC investigation. Immunohistochemically, MLH-1 (CLONE: ES05, Novocastra, 1/100 dilution, catolog number: MLH1-L-CE-S), MSH-2 (Clone 25D12, 1/100 dilution, catolog number: MSH2-CE-S), PMS-2 (Clone M0R4G, 1/100 dilution, catolog number: PMS2-L-CE-S) ve MSH-6 (Clone 25D12, 1/100 dilution, catolog number: MSH6-L-CE-S) primary antibodies were used. The positive control was nuclear staining in normal mucosa and/or lymphocytic infiltration.

At least 5 high power fields were evaluated for each tumor and the staining rate of the tumor cells was calculated. A mean percentage of stained tumor cells was determined and graded into three categories which is as follows: negative, < 1%; 1st group, 1-50%; 2nd group, 51-100% positive cells.

Statistical analysis

The statistical analyses were performed with the SPSS software version 19.0. “Kruskal-Wallis Test” and “Pearson Ki-Kare” were used. A P value ≤ 0.05 was considered statistically significant in all statistical analyses.

Results

There were 124 men (66.7%) and 62 women (33.3%). The mean age was 66.69 ± 11.90 years (range, 30-92 years). The numbers of

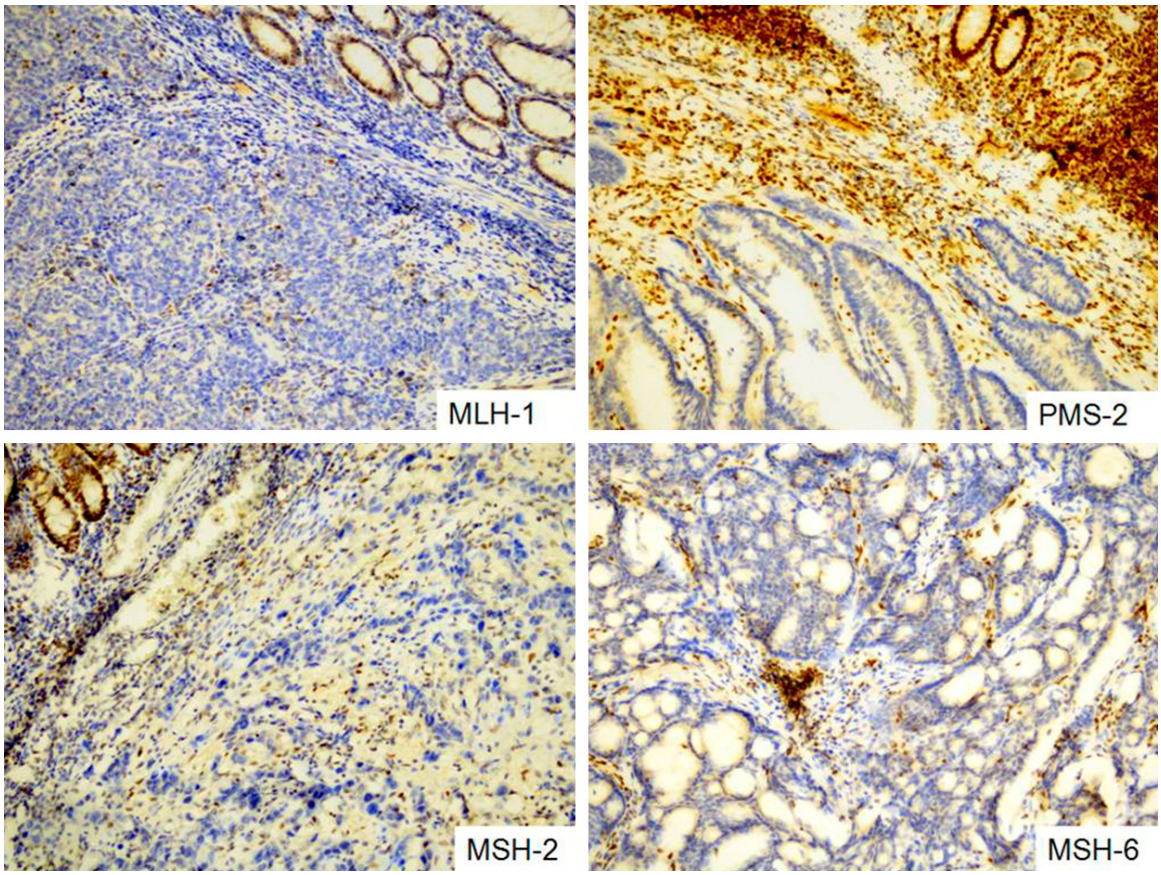

Figure 1. The immunohistochemical negativity for each one of four markers (MLH-1, x200; PMS-2, x200; MSH-2, x20; MSH-6, x200).

cases according to clinicopathological parameters are shown in **Table 1**.

Correlation between clinicopathological features

Statistically, right colon location was correlated with mucinous adenocarcinoma (P = 0.018) and presence of mucinous component (P = 0.001). The ulcerous tumors were smaller (P = 0.017) and their pathological tumor stage was higher (P < 0.0001). In the patients with ulcerous tumors, more lymph nodes were dissected (P = 0.012). In the tumors arising in polyp, size was bigger and the pathological tumor stage was lower (P = 0.008), and multiple tumors were more common than in the tumors not arising in polyp (P < 0.0001). Multiple tumors were together with polyps more frequently (P = 0.001) and their pathological lymph node stage was more advanced (P = 0.024). A statistically meaningful correlation was found between lymphocytic infiltration and accompanying polyp (P = 0.042).

In the poorly differentiated adenocarcinomas, mucinous component (P < 0.0001), lymphovascular (P = 0.001) and perineural invasion (P = 0.003) were more common. In addition, as the differention deteriorated, pathological tumor stage and lymph node metastasis increased (P= 0.001 and p = 0.019; respectively). Less lymphovascular invasion (P = 0.012) and lymph node metastasis (P = 0.002) were detected in the tumors containing mucinous differentiation. Tumors with lymphovascular invasion had more advanced pathological tumor stage (P = 0.001) and more lymph node metastasis (P < 0.0001). Perineural invasion was not detected in any of the pT1 and pT2 tumors. Statistically, perineural invasion correlated with surgical margin positivity (P = 0.021), lymh node metastasis (P < 0.0001), and pathological lymph node stage (P < 0.0001). As the degree of lymphocytic infiltration increased, the number of dissected lymph node increased, too (P = 0.030). Also, there was a statistically meaningful correlation between surgical margin positivity and regional lymph node metastasis (P =

Table 1. The numbers of cases according to clinicopathological parameters

	Number of cases	%		Number of cases	%
Age			Mucinous component		
<50	20	10.8	Absent	37	20.1
51-60	31	16.7	Present	134	71.5
61-70	61	32.8	Pathological tumor stage (pT)		
71-80	52	28.0	pT1	4	2.2
≥81	22	11.8	pT2	21	11.3
Sex			pT3	94	50.5
Male	124	66.7	pT4a	54	29.0
Female	62	33.3	pT4b	13	7.0
Localization			Lymphovascular invasion		
Cecum	15	8.1	Present	52	28.0
Ascending colon	22	11.8	Absent	134	72.0
Transverse colon	10	5.4	Perineural invasion		
Descending colon	17	9.1	Present	147	79.0
Sigmoid colon	69	37.1	Absent	39	21.0
Rectosigmoid junction	3	1.6	The lymphocyte density		
Rectum	50	26.9	Stromal lymphocytic infiltration	40	21.5
Growth pattern			Stromal lymphocyte infiltration and glandular infiltration	124	66.6
Ulcerous	38	20.4	Crohn-like lymphocytic infiltration regardless of the lymphocytic infiltration in the tumor	22	11.8
Ulcerovegetative	126	67.7	Tumor in surgical margin		
Polypoid	22	11.8	Present	16	9
Multiple tumors			Absent	170	91.0
Absent	172	92.5	The number of lymph node		
Present	14	7.5	≤ 12	31	16.7
Accompanying polyp			13-24	62	33.3
Absent	147	79.0	25-36	48	25.8
Present	39	21.0	37-48	24	12.9
Arising in polyp			≥ 49	21	11.3
Absent	173	93.0	Lymph node metastasis (pN)		
Present	13	7.0	pN0	91	48.9
Differentiation			pN1	46	24.7
Well	6	3.2	pN2a	20	10.8
Moderate	149	80.1	pN2b	29	15.6
Poor	17	9.1			
Mucinous	14	7.5			

0.016), perineural invasion (P = 0.021) and pathological tumor stage (P = 0.016). In our study, there was a statistically meaningful relationship between the number of dissected lymph nodes and lymph node metastasis (P = 0.049).

Correlation between immunohistochemical and clinicopathological features

For all four immunohistochemical markers (MLH-1, MSH2, PMS-2, MSH-6), loss of expression was correlated with poorly differentiated and mucinous adenocarcinoma histology (P < 0.0001, P = 0.015, P < 0.0001, P < 0.0001 respectively). Also, there was a correlation between localization to cecum and ascending colon and loss of PMS-2 and MSH-6 expression (P < 0.0001, P = 0.007 respectively). In the tumors with mucinous component, loss of MLH-1 was observed more (P = 0.003). Additionally, in the cases which exhibited loss of MLH-1 and PMS-2 expression, mucinous component (P = 0.003 and P < 0.0001), lymphovas-

Table 2. The number of the cases according to staining of the immunohistochemical markers

	< 1%	1-50%	51-100%
MLH-1	16 (8.6%)	52 (28.0%)	118 (63.4%)
MSH-2	3 (1.6%)	86 (46.2%)	97 (52.2%)
PMS-2	18 (9.7%)	26 (14.0%)	142 (76.3%)
MSH-6	7 (3.8%)	68 (36.6%)	111 (59.7%)

cular invasion ($P = 0.032$ and $P < 0.0001$) and intense intratumoral lymphocytic infiltration ($P < 0.0001$ and $P < 0.0001$) were observed more frequently. Half of the cases with loss of MLH-1 expression and two third of the cases with loss of MSH-2 expression are located to cecum and ascending colon. However, no statistical relationship was found between localization and MLH-1 and MSH-2 negativity ($P = 0.120$).

Correlation between immunohistochemical features

The number of the cases according to staining of the immunohistochemical markers is shown in **Table 2**. Immunohistochemically, in 2 cases, all four immunohistochemical markers were found negative. In 12 cases, staining was not observed with MLH-1 and PMS-2 while it was observed with the other markers. In 1 case, staining was not observed with MSH-2 and MSH-6 but it was observed with the other markers. PMS-2 and MSH-6 negativity was detected in 1 case. MLH-1 negativity alone was seen in 2 cases while PMS-2 negativity alone in 3 cases, just like MSH-6 negativity. The immunohistochemical negativity for each one of four markers is shown in **Figure 1** and the distribution of the number of the cases with negative immunohistochemical staining in **Figure 2**.

MSH-2 was negative in 2 of the 16 cases (12.5%) with no MLH-1 expression and in 1 of the 52 cases (1.9%) with 1st group MLH-1 staining. However, MSH-2 was positive in all of the 118 cases with 2nd group MLH-1 staining ($P < 0.0001$). No PMS-2 staining was detected in 14 (87.5%) MLH-1 negative cases, in three cases (5.8%) with 1st group MLH-1 staining and in one case (0.8%) with 2nd group MLH-1 staining ($P < 0.0001$). MSH-6 was negative in two (12.5%) MLH-1 negative cases, in three cases (5.8%) with 1st group MLH-1 staining and in two cases (1.7%) with 2nd group MLH-1 staining ($P < 0.0001$).

PMS-2 staining was not detected in 2 of the 3 cases (66.7%) with no MSH-2 expression and in 5 of the 86 cases (5.8%) with 1st group MSH-2 staining and in 11 of the 97 cases (11.3%) with 2nd group MSH-2 staining ($P < 0.0001$). MSH-6 was negative in all of the 3 cases with no MSH-2 expression, in 2 cases (2.3%) with 1st group MSH-2 staining and in 2 cases (2.1%) with 2nd group MSH-2 staining ($P < 0.0001$).

In 3 of the 18 cases (16.7%) with no PSM-2 expression; in 2 of the 26 cases (7.7%) with 1st group PSM-2 staining and in 2 of the 142 cases (1.4%) with 2nd group PSM-2 staining, MSH-6 staining was not detected ($P < 0.0001$).

Discussion

Colorectal cancer has the third highest cancer incidence in both sexes [1]. The mean age of incidence is 62, and it is rare under 40 unless there are predisposing factors [2]. The average of age in our study group complied with the literature and only 4.8% of the patients were under 40. It is reported that there is no noticeable sex dominance for CRCs; however, in the studies with a limited number of cases, the dominance can be tilted towards one sex as in our study [12, 13]. It is reported that CRCs are often localized in the rectosigmoid region but with age they move towards proximal colon and 2/3 of the tumors observed in Lynch Syndrome are localized to proximal colon [2, 12, 14, 15]. In most of the cases in our study, the tumor was localized to sigmoid colon and rectum. Although there was no statistical meaning, the rate of tumor localized to the right colon between the ages of 51 and 60 was slightly higher than in the other age groups. The reason why there is no statistical meaning may be that we have few patients under 40. The right colon tumors are often expected to be in an exophytic growth pattern, bigger and in more advanced stages while the left colon tumors are smaller and ulcerated [5-16]. In our study, in accordance with the literature, polypoid and ulcerovegetative tumors with exophytic growth pattern were mostly localized to right colon and bigger. Incidence of multiple CRC is 2.3-12.4% in the general population but that ratio is reported to be as high as 10-20% in the patients with FAP, Lynch Syndrome and ulcerative colitis [17]. As in our study, literature contains few studies reporting that the rate of lymph node metastasis is higher in multiple tumors [18]. In a study

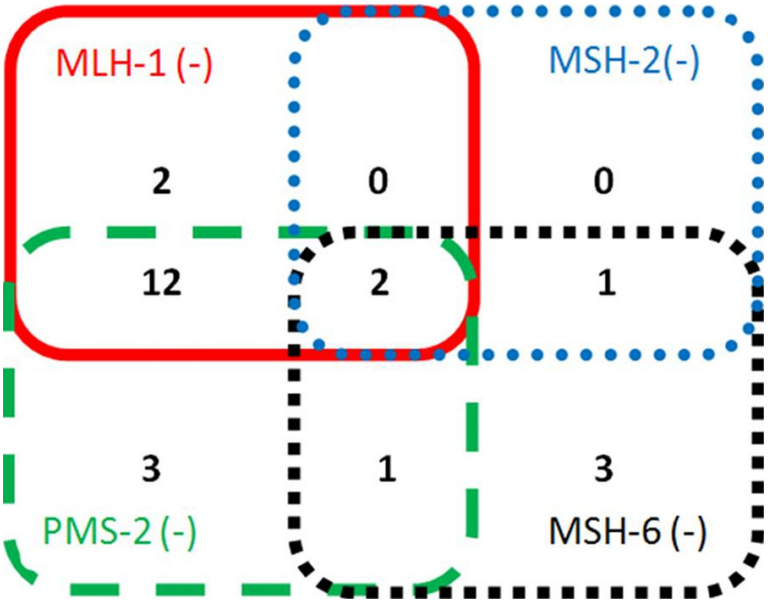

Figure 2. The distribution of the number of the cases with negative immunohistochemical staining and their togetherness.

of 42 patients with multiple tumors, conducted by Oya et al, it is claimed that the existence of multiple tumor focus can have a synergistic effect for distant metastases [17]. In our study, a correlation was found between multiple tumor focus and lymph node metastasis and this suggests that there could be a similar effect for lymph node metastases, a finding of locally advanced stage. In the cases with multiple tumor, presence of accompanying polyp and arising in polyp were found to be more common than in the cases with single tumor in our study. This result suggested that more synchronous tumors could occur in polyposis even if the number of polyps was low. Nevertheless, our finding must be supported with studies containing a large patient group and long term follow-up. There are studies which claim that histological grade alone is important as a prognostic factor independent of stage, and more perineural invasion, peritumoral lymphovascular invasion, lymph node metastasis and advanced tumor invasion are observed in higher grade tumors [2, 6, 7]. In our study, as in the literature, a positive correlation was detected between high grade tumors, lymphovascular and perineural invasion, lymph node metastasis and the depth of tumor invasion.

Mucinous adenocarcinomas are associated with young age, dominance of female sex, right colon localization, advanced pathological tumor stage, lymph node metastasis, poor differentiation and advanced clinical tumor stage [18-20]. When the mucinous adenocarcinomas and the tumors with mucinous differentiation in our study were evaluated together, most of them were found to be localized to the right colon and poorly differentiated tumors and they had lymph node metastasis in accordance with the literature. A relationship between morphological and macroscopic features mostly associated with MSI was also observed in our study. This finding suggested that our patients could have MSI although it was not confirmed with molecular methods.As known, as the depth of tumor invasion increases, the risk of lymphovascular invasion and lymph node metastasis increases, too [21, 22]. Besides, the lymphovascular tumor emboli outside the muscularis propria is reported to be a very strong prognostic factor [23]. In our study, in accordance with the literature, lymphovascular invasion was not seen in pT1 and pT2 tumors; all the patients with lymphovascular invasion had pT3 and pT4 tumors and as the depth of invasion increased, the incidence of lymphovascular invasion increased, too. Moreover, most of the patients with lymphovascular invasion had lymph node metastasis. Considering these findings, we think that if the pathological stage of the tumor is low in patients with peritumoral lymphovascular invasion, the depth of the tumor invasion must be examined with more sections. In the tumors with peritumoral lymphovascular invasion, even if the number of dissected lymph nodes is enough, if there is no lymph node metastasis, we concluded that recurrent dissections of the fat tissue are important in order to find the metastatic lymph nodes.

In 15-17% of the cases with colorectal carcinoma, microsatellite instability pathway is responsible for the pathogenesis [24]. In the autosomal dominant Lynch Syndrome arising in microsatellite instability pathway, the risk of developing metachronous and synchronous tumors at an early age increases [6]. The defini-

tive diagnosis of Lynch Syndrome can be made through genetic analyses and PCR-based MSI is recommended as the gold standard in choosing the patients to be referred for genetic analysis [25-27]. As for the CRCs which have microsatellite instability but are not familial, there are authors who suggest that screenings must be done because revealing the loss in proteins can be useful in determining the prognosis and response to treatment [25-28]. It is important to recognize the tumors with MSI in order to detect Lynch Syndrome and follow the other family members before cancer arises and to choose the treatment for patients. However, PCR-based MSI is costly and technically tricky so, for screening purposes, immunohistochemical examinations which may be used instead of PCR-based MSI have emerged [29, 30]. Under in vivo conditions, MMR proteins can be heterodimerized so protein loss can be isolated and it can be together with another MMR protein. Due to this situation posing difficulty in immunohistochemical evaluation, there are different studies about which protein the antibodies to be used in the screening must be for [30, 31]. In the immunohistochemical method, generally recommended basic monoclonal antibodies developed against DNA repair gene proteins are MLH-1, MSH-2, MSH-6 and PMS-2 [32, 33]. Immunohistochemically, if antibody negativity is considered as an indication of protein loss and if there are clinical and histopathological features expected to accompany, it is recommended that patients be referred to molecular examinations [14, 32].

Among the main clinical and histopathological features expected to accompany carcinomas with MSI are dominance of female sex, localization to right colon, tendency for multiple tumor, intense lymphocytic infiltration, mucinous or poorly differentiated carcinoma morphology [5, 12, 14, 25, 34]. Chapusot et al claimed that about one-third of tumors with MMR expression loss are localized to right colon and when localization to right colon is evaluated together with poorly differentiated adenocarcinoma morphology, it has a high predictive value for MMR loss [35]. In our study, in accordance with the literature, the cases who exhibited negativity with at least one of the immunohistochemical markers had right colon localization and poorly differentiated carcinoma morphology. A study conducted by Greenson et al suggests that not only mucinous adenocarcinomas but also the tumors with less than 50% mucinous component are associated with MSI [36]. In our study, in accordance with the literature, there was a meaningful correlation between the cases who exhibited negativity with at least one of the immunohistochemical markers and mucinous adenocarcinoma and/or mucinous component.

In most studies on colorectal carcinomas, lymphocytes infiltrating the tumor and the presence of Crohn's-like response were found to be associated with MSI [35, 37, 38]. In our study, in the tumors having intense lymphocytic infiltration, a meaningfully high negativity of immunohistochemical marker was observed, which is a finding in accordance with the literature. Clinically, CRCs having MSI are reported to be less likely to metastasize to local lymph node although they exhibit more advanced depth of tumor invasion [39]. In a study conducted by Raut et al, it is claimed that lymph node metastasis is less frequent in the cases with MMR loss [40]. However, what is striking in their study is that pN1c cases are regarded as non-metastatic. In our study, none of the immunohistochemical markers applied had a statistical relationship with the depth of tumor invasion or lymph node metastasis. In our study, however, pT1c cases were regarded to be metastatic according to the current WHO classification. Also, considering that sporadic MSI-H tumors have a more heterogeneous structure, it can be seen that the area studied does not represent the whole of the heterogeneous tumor in our study, where multiple tissue block was applied. We think that the connection between MSI and lymph node metastasis can be clarified better with studies having larger series and conforming to current classification. Due to the drawbacks of using PCR-based MSI test in screening familial cancer syndromes, immunohistochemical methods are gaining more popularity at the first step of the investigation. PMS2 and MSH6 followed MSH2 and MLH1, which were developed first [30, 31]. Because MMR proteins can be in vivo heterodimerized, it is thought that these four antibodies must be used together to increase the specificity and sensitivity [29, 31, 41]. In our study, focal staining was observed in part of the cases. When tumor heterogeneity and focal stainings are evaluated together, the immunohistochemical markers should, if possible, be used on the sur-

gical material, for better results, rather than on small biopsies. Also, in our study, in part of the cases with no staining, negativity was detected in one or some of the markers. When this feature detected in immunohistochemical stainings is considered, in accordance with the literature, it was concluded that these four best-known antibodies must be used together for high compliance to PCR-based germline mutation analyses. In order to follow the family members of the patient with Lynch Syndrome before cancer arises and to choose the right treatment for sporadic tumors, it is important to recognize microsatellite instability. Although the results of our study are not confirmed with PCR analyses, they show that immunohistochemical examination containing the antibodies MLH1, MSH2, PMS2 and MSH6 can be an appropriate step prior to molecular analyses if there are proper clinical and histopathological features. Our findings must be supported with studies containing molecular data and prognosis information in larger series.

Acknowledgements

We gratefully acknowledge Dr. Hatice Uluer in Department of Biostatistics and Medical Communication of Ege University for performing the statistical analyses.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Asuman Argon, Department of Pathology, Izmir Bozyaka Training and Research Hospital, Izmır 35400, Turkey. Tel: +902322505050; Fax: +902322502997; E-mail: asumanargon@gmail.com

References

[1] Siegel R, Desantis C, Jemal A. Colorectal cancer statistics, 2014. CA Cancer J Clin 2014; 64: 104-17.

[2] Rosai J. Gastrointestinal tract. In: Rosai J, editor. Rosai and Ackerman's Surgical Pathology. China: Elsevier Saunders; 2011. pp. 731-803.

[3] Lepisto A, Kiviluoto T, Halttunen J, Jarvinen HJ. Surveillance and treatment of duodenal adenomatosis in familial adenomatous polyposis. Endoscopy 2009; 41: 504-509.

[4] Labianca R, Beretta GD, Kildani B, Milesi L, Merlin F, Mosconi S, Pessi MA, Prochilo T, Quadri A, Gatta G, de Braud F, Wils J. Colon cancer. Crit Rev Oncol Hematol 2010; 74: 106-33.

[5] Kumar V, Robbins and Cotran. Pathologic Basis of Disease. In: Kumar V, Abbas AK, Fausto N, editors. Philadelphia PA: Elsevier Saunders; 2005. pp. 269-342.

[6] Boland CR. Evolution of the nomenclature for the hereditary colorectal cancer syndromes. Fam Cancer 2005; 4: 211-218.

[7] Bolocan A, Ion D, Ciocan DN, Paduraru DN. Prognostic and predictive factors in colorectal cancer. Chirurgia (Bucur) 2012; 107: 555-63.

[8] Worthley DL, Whitehall VL, Spring KJ, Leggett BA. Colorectal carsinogenesis: road maps to cancer. World J Gastroenterol 2007; 13: 3784-3791.

[9] Umar A, Boland RC, Terdiman JP, Syngal S, de la Chapelle A, Barrett JC, Rüschoff J, Fishel R, Lindor NM, Burgart LJ, Hamelin R, Hamilton SR, Hiatt RA, Jass J, Lindblom A, Lynch HT, Peltomaki P, Ramsey SD, Rodriguez-Bigas MA, Vasen HF, Hawk ET, Freedman AN, Srivastava S. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite ınstability. J Natl Cancer Inst 2004; 96: 261-268.

[10] Whitehall V, Leggett B. Microsatellite instability: detection and management in sporadic colorectal cancer. J Gastroenterol Hepatol 2011; 26: 1697-9.

[11] Yoon YS, Yu CS, Kim TW, Kim JH, Jang SJ, Cho DH, Roh SA, Kim JC. Mismatch repair status in sporadic colorectal cancer: immunohistochemistry and microsatellite instability analyses. J Gastroenterol Hepatol 2011; 26: 1733-9.

[12] Bosman T, Carneiro F, Hruban RH, Theise ND. World Health Organization Classification of Tumours of the Digestive System. 4th Edition. Lyon, France: IARC Press; 2010. pp. 131-181.

[13] Albasri A, Yosef H, Hussainy AS, Sultan SA, Alhujaily A. Histopathological features of colorectal cancer in Al-madinah region of saudi arabia: 8 years experience. Asian Pac J Cancer Prev 2014; 15: 3133-7.

[14] Michailidi C, Papavassiliou AG, Troungos C. DNA repair mechanisms in colorectal carcinogenesis. Curr Mol Med 2012; 12: 237-46.

[15] Mecklin JP. Frequency of hereditary colorectal carcinoma. Gastroenterology 1987; 93: 1021-5.

[16] Mark R. Epithelial neoplasms of the large intestine. In: Odze RD, Goldblum JR, Crawford JM, editors. Surgical pathology of the GI tract. Liver, Bilier Tract and Pancreas. Elsevier pres; 2004. pp. 441-472.

[17] Oya M, Takahashi S, Okuyama T, Yamaguchi M, Ueda Y. Synchronous colorectal carcinoma: clinico-pathological features and prognosis. Jpn J Clin Oncol 2003; 33: 38-43.

[18] Nitsche U, Zimmermann A, Späth C, Müller T, Maak M, Schuster T, Slotta-Huspenina J, Käser SA, Michalski CW, Janssen KP, Friess H, Rosenberg R, Bader FG. Mucinous and signet-ring cell colorectal cancers differ from classical adenocarcinomas in tumor biology and prognosis. Ann Surg 2013; 258: 775-783.

[19] Leopoldo S, Lorena B, Cinzia A, Gabriella DC, Angela LB, Renato C, Antonio M, Carlo S, Cristina P, Stefano C, Maurizio T, Luigi R, Cesare B. Two subtypes of mucinous adenocarcinoma of the colorectum: clinicopathological and genetic features. Ann Surg Oncol 2008; 15: 1429-1439.

[20] Greenson JK, Huang SC, Herron C, Moreno V, Bonner JD, Tomsho LP, Ben-Izhak O, Cohen HI, Trougouboff P, Bejhar J, Sova Y, Pinchev M, Rennert G, Gruber SB. Pathologic predictors of microsatellite instability in colorectal cancer. Am J Surg Pathol 2009; 33: 126-33.

[21] Sakai E, Takahashi H, Kato S, Uchiyama T, Hosono K, Endo H, Maeda S, Yoneda M, Taguri M, Nakajima A. Investigation of the prevalence and number of aberrant crypt foci associated with human colorectal neoplasm. Cancer Epidemiol Biomarkers Prev 2011; 20: 1918-24.

[22] Iacobuzio–Donahue CA. Epithelial Neoplasms of the Colorectum. In: Iacobuzio-Donahue CA, editor. Montgomery EA: Gastrointestinal and Liver Pathology. Pennsylvania: Churchill Livingstone; 2005. pp. 367-394.

[23] Silverberg SG, De Lellis RA, Frable WJ, Li Volsi VA, Wick MR. Neoplastic diseases of the small and large intestines. Silverberg's Principles and Practice of Surgical Pathology and Cytopathology. In: Silverberg SG, editor. Philadelphia: Churchill Livingstone Elsevier; 2006. pp. 1419-1464.

[24] Bartley AN, Hamilton SR, Alsabeh R, Ambinder EP, Berman M, Collins E, Fitzgibbons PL, Gress DM, Nowak JA, Samowitz WS, Zafar SY; Members of the Cancer Biomarker Reporting Workgroup, College of American Pathologists. Template for reporting results of biomarker testing of specimens from patients with carcinoma of the colon and rectum. Arch Pathol Lab Med 2014; 138: 166-70.

[25] Whitehall V, Leggett B. Microsatellite instability: detection and management in sporadic colorectal cancer. J Gastroenterol Hepatol 2011; 26: 1697-9.

[26] Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Nakagawa H, Sotamaa K, Prior TW, Westman J, Panescu J, Fix D, Lockman J, Comeras I, de la Chapelle A. Screening for the Lynch syndrome (Hereditary nonpolyposis colorectal cancer). N Engl J Med 2005; 352: 1851-1860.

[27] Umar A, Boland RC, Terdiman JP, Syngal S, de la Chapelle A, Barrett JC, Rüschoff J, Fishel R, Lindor NM, Burgart LJ, Hamelin R, Hamilton SR, Hiatt RA, Jass J, Lindblom A, Lynch HT, Peltomaki P, Ramsey SD, Rodriguez-Bigas MA, Vasen HF, Hawk ET, Freedman AN, Srivastava S. Revised bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite ınstability. J Natl Cancer Inst 2004; 96: 261-268.

[28] Yoon YS, Yu CS, Kim TW, Kim JH, Jang SJ, Cho DH, Roh SA, Kim JC. Mismatch repair status in sporadic colorectal cancer: immunohistochemistry and microsatellite instability analyses. J Gastroenterol Hepatol 2011; 26: 1733-9.

[29] Chubak B, Heald B, Sharp RR. Informed consent to microsatellite instability and immunohistochemistry screening for Lynch syndrome. Genet Med 2011; 13: 356-60.

[30] Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry. J Mol Diagn 2008; 10: 293-300.

[31] Shia J, Tang LH, Vakiani E, Guillem JG, Stadler ZK, Soslow RA, Katabi N, Weiser MR, Paty PB, Temple LK, Nash GM, Wong WD, Offit K, Klimstra DS. Immunohistochemistry as first-line screening for detecting colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome: a 2-antibody panel may be as predictive as a 4-antibody panel. Am J Surg Pathol 2009; 33: 1639-45.

[32] Amira AT, Mouna T, Ahlem B, Raoudha A, Majid BH, Amel H, Rachida Z, Nadia K. Immunohistochemical expression pattern of MMR protein can specifically identify patients with colorectal cancer microsatellite instability. Tumour Biol 2014; 35: 6283-91.

[33] Lindor NM, Burgart LJ, Leontovich O, Goldberg RM, Cunningham JM, Sargent DJ, Walsh-Vockley C, Petersen GM, Walsh MD, Leggett BA, Young JP, Barker MA, Jass JR, Hopper J, Gallinger S, Bapat B, Redston M, Thibodeau SN. Immunohistochemistry versusmicrosatellite instability testing in phenotyping colorectal tumors. J Clin Oncol 2002; 20: 1043-1048.

[34] Musulén E, Sanz C, Muñoz-Mármol AM, Ariza A. Mismatch repair protein immunohistochemistry: a useful population screening strategy for Lynch syndrome. Hum Pathol 2014; 45: 1388-96.

[35] Chapusot C, Martin L, Mungra N, Rageot D, Bouvier AM, Bonithon Kopp C, Ponnelle T, Faivre J, Piard F. Sporadic colorectal cancers with defective mismatch repair display a number of specific morphological characteristics: relationship between the expression of hMLH1 and hMSH2 proteins and clinicopathological features of 273 adenocarcinomas. Histopathology 2003; 43: 40-7.

[36] Greenson JK, Bonner JD, Ben-Yzhak O, Cohen HI, Miselevich I, Resnick MB, Trougouboff P, Tomsho LD, Kim E, Low M, Almog R, Rennert G, Gruber SB. Phenotype of microsatellite unstable colorectal carcinomas: Well-differentiated and focally mucinous tumors and the absence of dirty necrosis correlate with microsatellite instability. Am J Surg Pathol 2003; 27: 563-70.

[37] Ogino S, Nosho K, Irahara N, Meyerhardt JA, Baba Y, Shima K, Glickman JN, Ferrone CR, Mino-Kenudson M, Tanaka N, Dranoff G, Giovannucci EL, Fuchs CS. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. Clin Cancer Res 2009; 15: 6412-20.

[38] Ward R, Meagher A, Tomlinson I, O'Connor T, Norrie M, Wu R, Hawkins N. Microsatellite instability and the clinicopathological features of sporadic colorectal cancer. Gut 2001; 48: 821-9.

[39] Redston M. Epithelial Neoplasms of the Large Intestine. In: Odze RD, Goldblum JR, Crawford JM, editors. Surgical Pathology of the GI Tract, Liver, Biliary Tract and Pancreas. Pennsylvania: Churchill Livingstone; 2003. pp. 441-472.

[40] Raut CP, Pawlik TM, Rodriguez-Bigas MA. Clinicopathologic features in colorectal cancer patients with microsatellite instability. Mutat Res 2004; 568: 275-82.

[41] Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. Genet Med 2009; 11: 35-41.